U.S. Department of Agriculture Agricultural Research Service July 1993

Agricultural Research

Wheat: The Quest for Total Quality

Diversity Is Key to Biocontrol Success

Biological control has the potential to become the single most important approach to fighting plant pests and diseases in the 21st century.

To make this a reality, however, we must fully explore, exploit, and explain not only the familiar forms of biologically based control, but also new options presented by modern biotechnology.

We need beneficial insects such as the *Cephalonomia waterstoni* wasp noted in this issue (see "Pest Arrest in Manhattan, Kansas," page 7). But we also need to expand our vision of biocontrol to encompass bioengineered organisms and other biotechnological possibilities. Genetic engineers have already proven, for instance, that a fungus harmful to melons can be bioengineered to instead protect these plants from disease. We need to investigate thousands—or perhaps tens of thousands—of other biological control agents if we are to strengthen and deepen the ranks of the biocontrol army.

Why do we need such a vast diversity of species, biotypes, and strains of these agents, and their genes? Because there is strength in diversity. Ecologists recognize that the more complex the biological community, the less likely it is for any one organism, such as a pest, to dominate it. And biocontrol research suggests that different beneficial organisms will be needed to control the same pest in different crops or different environments.

For example, a helpful soil microorganism, *Agrobacterium radiobacter,* controls crown gall in crops like peach, apple, or rose. But it doesn't protect grapes against crown gall.

Too, plant breeding experience teaches us that different varieties are needed for different geographic regions. That's why we rely on hundreds of varieties of wheat and soybean, for instance.

But to amass a futuristic array of site-, disease-, and pest-specific biocontrol agents requires changes in science, finance, and regulation—and in attitudes.

Scientists must not reject prospective candidates on the basis that the range of effectiveness of these potentially useful organisms may be too narrow. In our hurry to discover biocontrols that are at least as wide-ranging as the target pest, we must not overlook small yet successful subpopulations of natural enemies.

Support for this research must be bolstered, perhaps by copying the model used for improving crops. Today's new crop varieties typically result from publicly supported plant breeding programs that turn over top-performing varieties to state crop improvement associations, growers' associations, seed producers, and seed companies.

Another strategy to increase private investment in biocontrol is to identify traits from the useful organisms that can be transferred to crops, thus creating potentially patentable disease- or pest-resistant plants.

Regulatory review and approval of promising biocontrol agents must be streamlined. Otherwise, many potential biocontrol agents could be cast aside—not because they would be of too little benefit to a user, processor, or consumer, but because of the prohibitive cost of providing data to regulatory agencies.

Users of biocontrol technologies must be willing to cope with strategies more complex and sophisticated than those associated with chemical control. Biological control is not a quick fix. It is more complicated, but also more enduring.

Users—and scientists—must also help a sometimes skeptical public understand the safeness and sound ecological foundation of biological control. Society no longer questions the need for hundreds or possibly thousands of species and strains of fungi to make favorite cheeses. We need similar acceptance of the tens of thousands of biocontrol agents we must recruit for tomorrow.

Only with public support and confidence can we expand the art and science of biological control and aid efforts to strengthen financing, simplify regulatory procedures, and ease adoption by users. Only then can we achieve biological control's full potential to give us new, safe, and sustainable means of protecting our crops and our environment.

R. James Cook
Agricultural Research Service
Pullman, Washington

Editor's note: R. James Cook's studies of promising microbial biocontrols for diseases that attack wheat roots is among the many accomplishments that recently won him election to the National Academy of Sciences, one of the highest honors afforded to American scientists and engineers.

Agricultural Research

Cover: Quietly ensconced in a mobile monitoring trailer, entomologist David Hagstrum eavesdrops on insect pests in grain storage bins at the U.S. Grain Marketing Research Laboratory in Manhattan, Kansas. Photo by Scott Bauer. (K5083-3)

Page 10

Page 16

Page 20

Vol. 41, No. 7
July 1993

Editor: Lloyd McLaughlin (301) 344-2514
Associate Editor: Linda McElreath (301) 344-2536
Art Director: William Johnson (301) 344-2561
Contributing Editor: Jeanne Wiggen (301) 344-2502
Photo Editor: John Kucharski (301) 344-2900
Assoc. Photo Editor: Anita Daniels (301) 344-2956

Agricultural Research is published monthly by the Agricultural Research Service, U.S. Department of Agriculture, Washington, DC 20250-2350.

The Secretary of Agriculture has determined that publication of this periodical is necessary in the transaction of public business required by law.

Subscription requests should be placed with New Orders, Superintendent of Documents, P.O. Box 371954, Pittsburgh, PA 15250-7954. Please see back cover for order form.

This publication was printed with soybean oil-based inks.

When writing to request address changes or deletions, please include a recent address label.

Address magazine inquiries or comments to:
The Editor, Information Staff, Room 408, 6303 Ivy Lane, Greenbelt, MD 20770

Mike Espy, Secretary
U.S. Department of Agriculture

R.D. Plowman, Administrator
Agricultural Research Service

Robert W. Norton, Director
Information Staff

Manhattan Lab Seeks Total Quality Wheat

Specialists in many fields work at delivering grain with precisely the right attributes.

Those who seek ways to improve our daily bread think the matter of wheat research is far too important to slice up in a piecemeal fashion.

At the U.S. Grain Marketing Research Laboratory at Manhattan, Kansas, researchers from a variety of scientific disciplines work on multiple projects toward a common goal: developing the technology for a "total quality grain marketing system."

"We need to be able to deliver, identify, and protect appropriate wheat quality at each step of the grain marketing system, from the genetic level through consumption," explains Virgil W. Smail, director of the Manhattan laboratory.

"The goal is to ensure a high-quality end product, such as a loaf of bread," Smail continues. "It's possible that in the future, wheat will be marketed based on objective end-use performance factors that would indicate the type of end product for which a wheat sample is best suited."

Today's loaf is the culmination of years of plant genetic studies and breeding, grain quality evaluation, and advances in crop pest management.

Anticipating changes in the marketplace, the Manhattan units specialize in plant science, entomology, grain quality and structure, engineering, and biological research. Each concentrates on finding ways to help develop and deliver wheat with the right blend of quality attributes for specific end-use markets.

Working projects together means some research efforts must be synchronized with others. For instance, an important part of the ARS research at Manhattan involves breeding grain to exclude pests and diseases, a painstaking process that can take many years. But while one unit breeds experimental plants for pest resistance [See "Pest Arrest in Manhattan, Kansas," page 7], another team of scientists extensively tests the new breed's baking characteristics.

"Screening experimental lines at the earliest possible generation helps increase the efficiency of breeding programs and ensure that good wheat is being produced," says chemist Okkyung Kim Chung, who is in charge of the Manhattan lab's Grain Quality and Structure Research Unit.

SCOTT BAUER

Visiting with baking technologists from the American Institute of Baking, ARS technicians Margo Caley (left) and Bernadine Eichman (second from right) exchange information and evaluate samples of freshly baked test loaves. (K5080-12)

In addition to coordinating research projects, another set of considerations needs to be factored in: the concerns of industry.

"We're in constant touch with milling and baking industry representatives and other end users, to be certain that the desired attributes are maintained," adds Chung.

Chemist George L. Lookhart uses a variety of analytical methods to identify various types—cultivars—of wheat. Among these methods is electrophoresis. Electrically charged particles—usually protein—are pulled through a gel by an electric field that is similar to the pull of a magnet. Staining the proteins in the gel results in a "fingerprint" that helps researchers distinguish between cultivars.

"Proteins are a major contributing factor to baking quality," says Lookhart. "Once a cultivar is identified by its protein fingerprint, end users have an indication of potential product quality based on previous performance of that cultivar."

Other rapid analytical methods are being developed and used to determine wheat components such as lipids and starch types that are necessary for high-quality baked goods.

To Bake a Better Loaf

The ultimate test of the flour that a wheat breed makes, not surprisingly, is still to bake it into loaves of bread. In the quality and structure unit at Manhattan, scientists bake small loaves of bread from the various wheats. These pup loaves, as they are called, help scientists predict the baking quality of advanced hard winter wheat lines before seed is made available to growers.

Even after sample loaves are baked, one more point of view needs to be integrated into a total quality grain marketing system: quality control.

As grain moves out of the bin and into marketing channels, buyers need to be as-

sured that what they actually receive is what they agreed to purchase. Herein lies the need for rapid, accurate quality measurement systems, says James L. Steele, head of the Engineering Research Unit.

"Classification of wheat has always been done by sight," says agricultural engineer Charles R. Martin. "But partly because breeders have begun crossing classes of wheat such as hard and soft wheats to maximize desirable traits, visual classification has become increasingly difficult."

To help remedy the problem, in 1985, Congress ordered USDA's Federal Grain Inspection Service (FGIS) to develop an objective system of wheat classification.

Near-infrared reflectance was studied, but it could not distinguish between individual hard and soft kernels in a bulk wheat sample or determine if a ground sample contained a mixture of the two wheat classes.

After considering two other single-kernel hardness testers, Manhattan's single kernel wheat characterization system was selected for field testing in late 1990.

This system, invented in the grain lab's Engineering Research Unit, will be used to determine wheat hardness and classification.

Because different types of wheat have various end uses, it is important to separate hard and soft wheat during marketing. For example, hard red winter wheat is best for bread, while soft red winter wheat is more useful in making cookies, crackers, and pastries.

The instrument and its singulator—individual kernel separator—are patented. The inventors are Martin, Robert Rousser, and Daniel L. Brabec.

"The singulator delivers the grain to the tester, where it is weighed and then crushed to determine kernel moisture, size, and hardness," says Martin. Hardness is corrected for the effect of moisture, weight, size, and temperature. The instrument tests about 90 kernels a minute.

Through a cooperative research and development agreement with Perten Instruments North America, Inc., Steele, Martin, and their colleagues built two commercial prototypes that have been accepted by ARS and evaluated by FGIS. After conducting field tests, FGIS expects to begin using the commercial version for official wheat classification in 1995.

Another effort by the Engineering Research Unit to find objective measures to grade wheat focuses on digital image analysis.

Digital imaging is a computerized method of converting pictures into numbers. Inna Y. Zayas, an electrical engineer, is testing digital imaging for use as a grain grading method. The ultimate goal is to completely automate the U.S. grain grading and classification system.

"Image analysis has great potential, but there are several hurdles to clear before it can be used successfully," says Zayas.

Using image analysis of the shape and size of wheat kernels in combination with crushing-hardness scores, about 94 percent of hard and soft wheat was correctly classified. Image analysis can also distinguish between hard and soft wheat after it is ground.

SCOTT BAUER

Technician Margo Caley (nearest camera) mixes dough samples while technician Bernadine Eichman removes pup loaves from an oven. (K5079-07)

SCOTT BAUER

A new, automated system demonstrated by electrical engineer Inna Zayas uses digital imaging to evaluate the texture of grain products. (K5070-06)

Zayas and Donald B. Bechtel, a chemist in the quality and structure unit, have found that it is possible to use digital imaging to distinguish starch grains of hard red winter from those of soft red winter wheats.

And Zayas is developing digital imaging for analysis of crumb texture of bread slices. This test may determine if a slice of bread made from wheat samples being tested by Manhattan scientists meets the end-use quality limits set by the baking industry.

The Nose Knows

Other efforts to automate grain grading are taking the form of a mechanized nose. This device may one day eliminate the need for inspectors to actually sniff dozens of grain samples each day.

"Grain can pick up odors from molds, bacteria, insects, chemical sprays, and the like," says Larry M. Seitz, a chemist in the quality and structure unit. "The potential problem is that these odors could be passed on to flour, cereal and bakery products, and animal feeds."

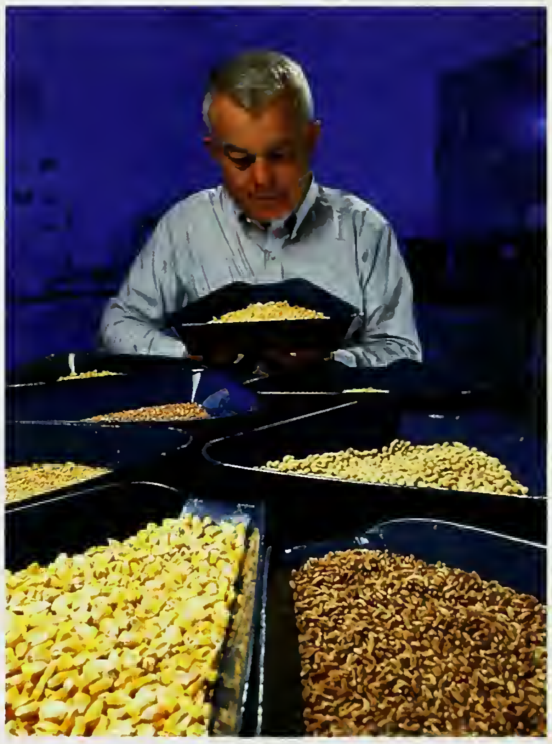
Official FGIS and state inspectors trained to detect off-odors in grain regularly sniff samples to ferret out the bad-smelling grain. Current procedures call for the inspectors to simply put their noses in a sample and take a whiff. However, not everyone can detect the same odors.

"Individuals vary in their sensitivity to certain odors and overall perception of smells," says Seitz. "If a person smokes or has a cold, there may be a problem identifying certain odors."

At the request of the grain inspection service, Seitz and plant pathologist David B. Sauer are matching human descriptions of off-odors found in grain with gas chromatograph analyses of these odors.

Plans are to include the information in a database of chemicals associated with known odors to give the grain industry a quick, simple, objective method to assess grain odors.

SCOTT BAUER



To build a database linking characteristic odors with various compounds, chemist Larry Seitz first sniffs grain samples and then analyzes them with gas chromatography. (K5071-13)

The scientists envision that a mechanical nose would be built around a gas chromatograph unit, but say that it may be several years before the technology is simplified and ready for use by FGIS.

Many of these and other quality prediction and preservation technologies must be expanded and adapted for use in wheat breeding programs, on-farm grain storage systems, elevators, mills, and bakeries as an integrated grain quality maintenance system for the future.

"The goal is to use the various technologies developed here at the U.S. Grain Marketing Research Laboratory and by other ARS wheat quality research efforts to create an integrated quality-based market system for U.S. grain for the next century," says Smail.—By **Marcie Gerriets,** ARS.

Scientists mentioned in this article are with the U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502. Phone (913) 776-2702, fax number (913) 776-2792. ◆

Acoustic Sensors Detect Insects Deep Inside Grain Bins

Inspectors now visually inspect grain to see if it's infested, but they don't always see the insects. So researchers say listening may be a more effective approach.

They're using acoustic sensors to listen to the sounds of beetles moving in the wheat inside grain bins, says David W. Hagstrum, an entomologist with USDA's Agricultural Research Service in Manhattan, Kansas. He heads a cooperative project with Dennis Shuman, an ARS electrical engineer in Gainesville, Florida.

Researchers in Manhattan and Gainesville have begun a 3-year study to monitor wheat stored at two farms near Manhattan.

To pick up insect sounds, researchers strung seven cables vertically inside two bins on each farm. Attached to each cable are 20 sensors placed 6 inches apart. The 140 sensors in each bin are hooked to computers that monitor insect sounds. Hagstrum says one objective of the pilot study is to determine how many sensors are

SCOTT BAUER

In a soundproof room, entomologist David Hagstrum positions acoustic sensors in a scale model grain bin made from a 55-gallon barrel. (K5081-04; K4244-17)

needed in each bin to accurately monitor the grain.

The sensors let scientists know where the insects are based on which sensor is picking up the feeding sounds of the lesser grain borers, rice weevils, and other stored grain pests present.

"Farmers will easily know how severe an infestation they have, or if their grain is still free of insects," says Hagstrum.

This will let them know when to use the right amount of chemical fumigants for control, and it is "a big step forward from the way stored grain is monitored now," he adds.

David W. Hagstrum is at the U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502. Phone (913) 776-2718, fax number (913) 776-2792.

Dennis Shuman is at the USDA-ARS Insect Attractants, Behavior, and Basic Biology Research Laboratory, P.O. Box 14565, Gainesville, FL 32604. Phone (904) 374-5737, fax number (904) 374-5781.

SCOTT BAUER

Pest Arrest in Manhattan, Kansas

The American grain industry, listening carefully to its domestic and overseas customers, admits there's a challenge out there. The challenge is to meet the ever-more-exacting demands of today's wheat market at a time when chemical pest controls alone are clearly not the answer. Instead, scientists are drawing upon greater resourcefulness and creativity than ever as they step up their struggle against some of agriculture's oldest (and smallest) enemies—insects and microorganisms that invade our crops.

Many of these innovative efforts are in progress at the U.S. Grain Marketing Research Laboratory in Manhattan, Kansas.

SCOTT BAUER

A biological control, *Bacillus thuringiensis*, sprayed by technician Richard Hammel on the surface of stored wheat kills Indian meal moth larvae that eat the grain. (K5077-02)

Saying No to Granary Insects

Put even a small amount of wheat in storage, and an assortment of insect pests is going to come to call.

Grain producers can fend off some storage pests with a natural soil bacterium called *Bacillus thuringiensis* (Bt), which has been used against crop pests for more than 30 years. The bacterium forms crystalline proteins that contain an array of insect toxins that are sprayed on stored grain to ward off the pests.

William H. McGaughey heads the Biological Research Unit at Manhattan, which focuses on stored grain insects. A team is now studying Bt's toxic proteins and how they affect target insects.

"Each variety of Bt produces a unique array of toxins that determines which pest insects it will control," explains McGaughey. "But we are finding that insects can become resistant to Bt and survive on treated grain."

So McGaughey and colleagues are tracking down the mechanisms that determine the specificity of the toxins and enable pests to adapt to the Bt toxins. Once they understand these mechanisms, the scientists hope to engineer Bt strains that will be toxic to a wider range of insects and more resistant to insect adaptation.

Others in the research unit are looking at the biological, physiological, and genetic makeup of insects—clues that will help in development of alternative pest control methods.

ARS chemist Karl J. Kramer and molecular biologist Brenda Oppert, along with Thomas D. Morgan, a technician, have found that certain proteins cause a vitamin deficiency or inhibit the digestive enzymes of stored grain pests such as the red flour beetle and the European corn borer.

"In effect, these proteins give the pests a severe stomach ache and eventually kill them," says Kramer.

That work, conducted in cooperation with Kansas State University and the Des Moines-based seed company Pioneer Hi-Bred International, focuses on combining several of these natural compounds to maximize their effect on the insect's gut.

One goal is to transfer the genes responsible for the insect growth-inhibiting compounds into crops. Crops containing these genes would appeal to pests less but would not affect human and livestock digestion.

Avidin and streptavidin, two proteins that bind to biotin, or vitamin H, may also inhibit stored grain pests by interfering with their nutrition.

"Without biotin in the diet, insects cannot grow and develop properly," Kramer says. "Of all the potential insecticidal proteins tested so far, the biotin-binding ones show the most promise."

They plan eventually to incorporate the avidin or streptavidin gene into plants and test to see whether the plants resist the insects.

Another approach to protecting stored grain is to find ways to weaken the outer covering of an insect—the exoskeleton—so that it cannot survive in the stored grain.

Kramer and Kansas State University colleague Subbaratnam Muthukrishnan note that specific natural enzymes enable insects to molt.

"Our long-term goal is to control stored-grain insects by manipulating either molting enzyme activity, the expression of the genes responsible for molting, or both," says Kramer.

Another potentially useful genetic tool is a maternally acting lethal genetic system discovered in 1991 by ARS entomologist Richard W. Beeman and KSU colleague Rob Denell.

In this system, if a certain gene is carried only by the male insect parent and passed to its offspring, that gene does not affect offspring survival. But if the gene is carried only by the female parent insect, those offspring that do not inherit the gene die. [See "Maternal Gene Life or Death for Flour Beetle," *Agricultural Research*, January 1993, p. 23.]

The scientists are hopeful the gene may one day be useful as a biological control agent. Biocontrols such as these are becoming increasingly important as the use of synthetic organic chemicals is restricted.

Pilot-Scale Elevator Unique in USDA Research

In other studies by the engineering unit, the Manhattan lab's grain quality elevator facility is used to develop and test new procedures for reducing grain damage through better drying and handling procedures. The pilot-scale elevator located at Manhattan is the only one in the USDA system.

SCOTT BAUER

Chemist Karl Kramer (left) and technician Leon Hendricks use the tobacco hornworm as a model for testing an experimental insect control protein. (K5073-01)

"The major concerns in long-term grain storage are insect and fungus damage, which are highly dependent on grain temperature and moisture," says agricultural engineer Cheng S. Chang.

Having available the means to predict when grain temperature and moisture will become a problem would help grain handlers know when to aerate or fumigate. And keeping grain uniformly cool and dry is one of the best nonchemical means there is to keep it clean and edible.

The Manhattan grain elevator is also used to develop and test more gentle ways to move and handle grain. Breakage during high-volume handling is a major quality concern for all grains, especially for corn.

Computer Programs Help Make Bug-Control Decisions

Managers of stored grain facilities will soon be getting help from experts who are writing programs that can decide if and when chemical treatment is needed.

One such program, called an expert system, mimics the problem-solving ability of a human expert—in this case, a manager of a stored grain facility. It's been designed to predict what is likely to happen in grain bins under different storage conditions.

The computer program uses models based on several variables to forecast insect population growth in stored grain.

Users provide information such as the planned storage duration, initial grain temperature and moisture, and whether the grain will be aerated. In return, the expert system advises how long the grain can safely be stored. It may also alert a manager to potential problems and suggest changes to avoid or limit insect infestations.

"The expert system can predict storage problems specific to each situation," says ARS biologist Paul W. Flinn, who developed the system in collaboration with David W. Hagstrum, an entomologist.

The expert system is also designed to help farmers or grain managers identify insect pests that may be prowling their grain bins. It inquires about the size of the insect, body color, and other physical attributes. Based on the responses, the system narrows the field of possible culprits to a few insect species and displays a picture of the probable pest on the computer screen.

The program also provides information about how to sample grain for insects and sets thresholds to determine when enough insects are present to require fumigation.

"A person can really learn a lot about grain storage management just by using this system," Flinn says.

The system is currently being field-tested to check the accuracy of its predictions and recommendations, and it's being adapted for use by commercial grain storage facilities. Although the programs were written for stored wheat, a few minor changes will make it useful for other grains.

Flinn says the expert system, which runs on either Macintosh or MS-DOS computers, will be available by early 1994. Plans are to make the program available through ARS and possibly local Cooperative Extension Service offices at minimal cost.

Meanwhile, the Manhattan scientists are also gathering information about stored grain pests' feeding habits by listening to the insects as they munch their way through grain kernels. [See box.]

Data from acoustic sensors may eventually be fed into the expert system to allow more accurate predictions of safe storage life, says Hagstrum, who heads the research project. Hagstrum and Flinn are working to couple the acoustic sensing system with the expert system.

Breeding Problems Out

For scientists who breed wheat, the first line of defense against pests begins long before a variety is ever planted.

In the Plant Science and Entomology Research Unit located on the campus of nearby Kansas State University, Merle G. Eversmeyer leads a team of researchers who develop new hard red winter wheat germplasm—types of wheat that carry natural resistance to the Hessian fly, leaf and stem rust, and other pests.

It's an effort that requires patience, for it can take as much as 15 years of breeding to produce a new wheat variety for release to growers. But the effort pays off when it yields a healthy wheat that delivers a high-quality kernel to the marketplace.

One member of Eversmeyer's group, ARS entomologist J.H. Hatchett, has conducted cooperative research with KSU scientists showing that x-rays will break wheat and rye chromosomes. This key discovery enables genes from rye—which is highly resistant to Hessian fly—to attach to wheat chromosomes. [See "Wheat Tough Enough To Take the Hessian Fly," *Agricultural Research,* September 1991, pp. 22-23.]

The result: wheat that can foil the hungriest Hessian fly.

Public and private plant breeders can obtain seed for use in breeding this valuable trait into commercial wheat varieties from the Wheat Genetics Resource Center, Department of Plant Pathology, at Kansas State University.

Another notorious enemy of top-quality, top-yielding wheat is leaf rust, a fungal disease that attacks wheat and hinders seed formation, lowering yields. The disease can also depress protein content.

ARS wheat breeders have found ways to endow plants with genetic resistance to leaf rust. Genes from three subspecies of a type of wild wheat, *Triticum monococcum,* "appear to be very effective against rust," notes Thomas (Stan) Cox, a wheat geneticist in the Plant Science and Entomology Research Unit. Working with KSU scientists, Cox crossed *T. monococcum* with common wheat, creating three new breeding lines with the desired genes.

One of the lines has already been released to plant breeders; the remaining two with rust resistance will undergo further testing before release.

The rust-resistant line was the eighth wheat germplasm released to wheat breeders since 1986 by the ARS-KSU joint project.

But not all insects are bad news, the scientists note. Beneficial insects such as parasitic wasps may one day help grain managers control infestations.

One such wasp, *Cephalonomia waterstoni,* sometimes already found in small numbers in grain bins, moves through a storage facility looking for beetles to parasitize, much like a detective might stalk and capture a suspected criminal.

Ralph W. Howard, an ARS chemist, wants to know just how these beneficial wasps track down their victims and how they know when they've found the right one.

Grain beetle larvae feed on the inside of a wheat kernel and emerge when they're ready to pupate or form a cocoon.

But before the larvae settle into the pupal stage, they wander through the grain mass looking for a place to cocoon, leaving a faint chemical trail behind. The wasp follows one of these trails until it finds a larva, paralyzes it with a sting, and then lays eggs on it.

"It appears that the beetle unwittingly leaves this trail that ultimately leads to its demise," Howard says.

Flinn and Hagstrum are developing a method to mass-rear the wasps and make their use in grain facilities a reality.—By **Marcie Gerrietts,** ARS.

Merle Eversmeyer, J.H. Hatchett, and Stan Cox are in the USDA-ARS Plant Science and Entomology Research Unit, Throckmorton Hall, Kansas State University, Manhattan, KS 66506. Phone (913) 532-6168, fax number (913) 532-5692. Other scientists mentioned in this article are with the USDA-ARS U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502. Phone (913) 776-2702, fax number (913) 776-2792. ◆

Weed-Eating Insects Take the Starthistle Challenge

A team of tiny but tenacious insects might someday stop the spread of a poisonous weed, yellow starthistle.

Imported from Greece, the diminutive weedeaters are three kinds of hardworking weevils and one species of delicately colored fly. Each would easily fit on the tip of your little finger.

The insects' offspring—grub or maggotlike larvae when young—are seed-destroyers that feast in starthistle's flowerhead, where seeds are formed. They vary in when and how they attack. Some larvae eat developing seeds. Others destroy tissue that otherwise would nurture young seeds.

"Without seeds, starthistle can't reproduce," says ARS researcher Charles E. Turner at Albany, California. "With this team of flowerhead feeders, we hope to slow or stop the spread of starthistle."

The insects offer an environmentally friendly alternative to using herbicides. This type of biological control has proven successful with other weedy pests in the United States and worldwide. A classic example: About 40 years ago, an imported beetle took only 5 years to knock down the northern California infestation of another poisonous plant, klamathweed.

At its worst, however, klamathweed probably infested no more than 2 million acres of California rangeland. Yellow starthistle is a more formidable foe. Introduced here in the 1800's, probably from southern Eurasia, starthistle now grows in 23 states. For four of them—California, Oregon, Washington, and Idaho—the weed has become a major pest. In California alone, starthistle now infests some 8 million acres.

In summer, a fully grown starthistle can measure from 6 inches to 6 feet high. Beneath its bright yellow flowers, needle-like thorns form a star-shaped circlet that gives the weed its name.

A starthistle toxin causes a chewing disease, nigropallidal encephalomalacia, in horses. Unable to chew or swallow, poisoned animals may die of thirst or starvation. Too, the nasty weed crowds out native plants and other greenery that could be safely grazed by horses, cattle, or sheep. And its painfully sharp thorns stab unwary hikers.

In a northern California pasture invaded by yellow starthistle, botanist Charles Turner releases *Larinus curtus* weevils in their first-ever U.S. test.

Since 1984, ARS scientists in the United States and colleagues Rouhollah Sobhian, Luca Fornasari, and others at the ARS European Biological Control Laboratory, Montpellier, France, have systematically recruited the five insect species that make up today's American team of weed warriors.

Each species has undergone intensive study and testing: The scientists had to find out as much as they could about each potential new recruit. That included checking to make sure each candidate species attacks only starthistle—not native plants or crops like safflower, sunflower, or artichoke—three starthistle relatives. The scientists used these tests results, and others, in seeking federal and state approvals to set the recruits free in the West.

Right now, the most numerous of the immigrants is *Bangasternus orientalis*, a dark-brown, quarter-inch-long weevil with a funny-looking snout.

In 1985, ARS scientists in Europe hand-collected *B. orientalis* to turn loose in three thistle-infested sites in California. Surrounded by a sea of food and a mild climate reminiscent of their Mediterranean home, the weevils flourished. Counties that were the insect's first California home soon became nurseries, providing new generations of healthy, hungry weevils. Today, more than 45 California counties have their own colonies of the little weevil.

Turner credits *B. orientalis* and other insects overseas with keeping the weed in check in its homelands. "In Europe, you can easily find many different kinds of insects living on a single starthistle plant," he says. "You'll see individual plants here and there, and some occasional thistle patches. But there's nothing like the vast infestations of starthistle that we have here.

"These teams of insects are apparently holding the weed back. And that's what we want them to do for us."—By **Marcia Wood,** ARS.

Charles E. Turner is with the USDA-ARS Western Regional Research Center, Plant Protection Research Unit, 800 Buchanan St., Albany, CA 94710. Phone (510) 559-5975; fax number (510) 559-5777. Rouhollah Sobhian and Luca Fornasari are at the USDA-ARS European Biological Control Laboratory, BP 4168, Agropolis, 34092, Montpellier, Cedex 5, France. Phone 33 67 04 56 00, fax number 33 67 04 56 20. ◆

Watching Out for Honey Bees

Lab Service Helps Protect Valuable Insect Pollinators

How would you feel if you opened your mail and found dead honey bees inside? Some kind of practical joke, you might think.

At the Bee Research Laboratory in Beltsville, Maryland, it happens on a regular basis—and the scientists there enjoy opening the packages of bees that arrive wrapped in newspaper or paper towels, or immersed in containers filled with alcohol. By examining those bees, researchers can see which problems are affecting honey bees.

But, occasionally, something else comes wrapped with the bees. Several years ago, researchers unwrapped newspaper to find a chicken head inside, along with dead bees implicated in the animal's death.

"It was beginning to rot because it wasn't preserved in alcohol," explains Hachiro Shimanuki, who heads the Agricultural Research Service lab where examining dead bees is part of a normal day's work.

The mailing, it turns out, came from a Missouri farmer who was convinced that the aggressive and much-publicized Africanized honey bees had attacked and killed one of his chickens. Since the bee lab is widely known for investigating these sorts of mysteries, the farmer sent the evidence there.

"We don't know the cause of the chicken's death, but we do know that the bees weren't Africanized," says Shimanuki. "They were European. But what killed the chicken, that's anyone's guess. The same goes for bees. We look for things that were affecting the bees, although we can't always determine what caused their death. You might say we never see the patient; we only see the body."

It's a dangerous world out there for honey bees. Mites, diseases, pesticides—a honey bee could fall victim to any of them, not to mention being swatted to death by someone fearing a sting. If the typical worker bee avoids these dangers, it will live for about 6 weeks in the summer after working almost nonstop, foraging for the honey bee's two main food sources: nectar and pollen.

SCOTT BAUER

Entomologist David Knox and microbiologist Hachiro Shimanuki assess winter damage to a colony of honey bees. (K5126-04)

But many bees don't make it to old age. Shimanuki and other researchers at the lab see the victims of these threats almost every day. Since 1907, the lab has been providing what he calls a diagnostic testing service for honey bees.

Each year, the lab receives about 2,000 samples of bees that have died from various causes. While the scientists may not always know what killed the bees, they are skilled at finding out what ailed them when they died.

Most samples arrive during the peak spring period of April, May, and June, when bees actively forage for food after being inside during winter. About two-thirds of them come from state bee inspectors who are trying to confirm that a particular colony died of a certain disease or from some other cause. The remaining samples come from beekeepers or others who want to know what happened to a particular honey bee—or, in rare cases, what happened to a particular chicken.

"We look at it as our link to what's happening out there," says David A. Knox, an entomologist in the lab. "It lets us know if any new diseases or other problems are developing that we need to know about."

Why be concerned about dead insects? If honey bees were pests—like mosquitoes, corn earworms, or the sweetpotato whitefly—researchers would be looking for ways to kill them. But honey bees are nature's most reliable insect pollinators. Each year they pollinate billions of dollars in crops, so threats to their well-being are taken seriously. Their behavior, foraging patterns, communication, honey production, and many other attributes have been researched at great length. They're probably the most inspected, regulated, and studied of all insects.

Over the years, scientists have seen changes in the diseases and pests that threaten honey bees. "We've been able to notice trends because of the samples

SCOTT BAUER

Hachiro Shimanuki, supervisor of the bee disease diagnostic lab, examines a honeycomb sample for wax moth damage. (K5128-08)

we receive from all over the country," Shimanuki says. "We were able to confirm when *Varroa* and tracheal mites came into the United States in the mid-1980's. If we hadn't had this diagnostic program, it might have taken much longer for us to determine that." [See "Fighting for Survival Against Bee Mites," *Agricultural Research,* March 1993, pp.14-17.]

The program also helped Shimanuki and colleagues discover that the fungus that causes chalkbrood disease also produces a substance that inhibits the growth of the bacterium that causes European and American foulbrood diseases.

From the early 1960's to 1988, researchers studied ways to control European foulbrood in southern New Jersey, where the disease was widespread in areas where honey bees were used to pollinate blueberries and cranberries. European foulbrood, caused by the bacterium *Melissococcus pluton*, is a serious disease that infects and kills honey bee larvae.

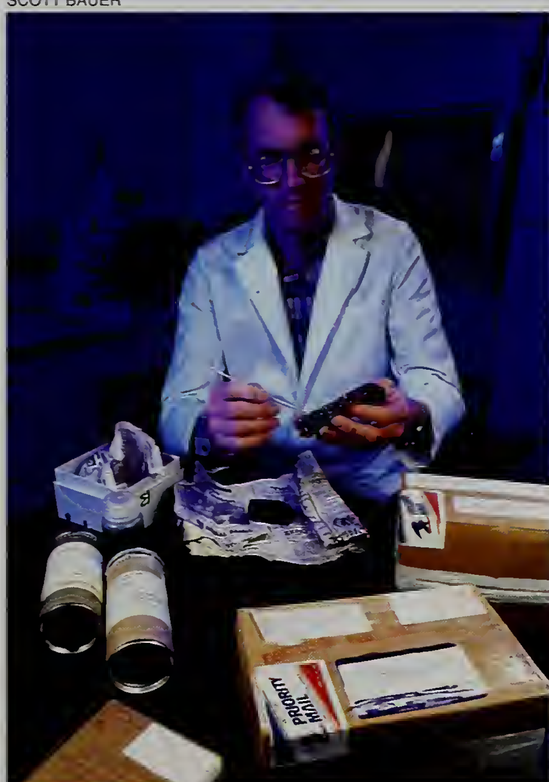
At the same time, Shimanuki says, researchers also discovered an increase in chalkbrood, caused by the fungus *Ascosphaera apis*. A disease that infects bee larvae, it had become widespread in southern New Jersey by 1983.

But samples coming into the lab showed an interesting trend. During 1980-90, bees from New Jersey analyzed at Beltsville showed a drastic decline in European foulbrood—to the point where it was difficult to find the disease in New Jersey by the late 1980's. Meanwhile, he says, the number of chalkbrood samples analyzed at Beltsville remained constant.

Further tests confirmed that the *A. apis* fungus produced a substance that inhibited not only the European foulbrood bacterium, but also the *Bacillus larvae* bacterium that causes American foulbrood, a serious disease that can kill bee larvae if unchecked. Shimanuki says American foulbrood is the second biggest threat to honey bees today, after mites.

Shimanuki and Knox, along with entomologist Mark F. Feldlaufer and chemist William R. Lusby of the ARS Insect Neurobiology and Hormone lab in Beltsville, have isolated the protective substance from *A. apis* and are filing a patent application on their discovery. The researchers say it could provide the basis for a new, effective, and inexpensive control for American and European foulbrood. Now, in the United States, only the antibiotic oxytetracycline is approved by the Food and Drug Administration for the control of both foulbrood diseases. Shimanuki says, "There's always the danger that these foulbrood diseases could develop resistance to oxytetracycline, so we need new controls."

SCOTT BAUER



Every year, entomologist David Knox and other ARS Bee Research Laboratory staff handle about 2,000 inquiries. (K5127-01)

The bee disease I.D. program has helped the lab develop methods for diagnosing foulbrood diseases and others, he says, and led to ARS publication in 1991 of a USDA handbook, "Diagnosis of Honey Bee Diseases." It's designed to assist inspectors who diagnose and treat honey bee diseases, and the lab has trained visiting researchers and inspectors from as far away as India, Jamaica, New Zealand, and Trinidad.

And the program alerts scientists to new pests and diseases. For example, a mite called *Tropliaelaps clareae*, indigenous to Southeast Asia, is not found in the United States but could be devastating if it showed up here. But ARS scientists in the Beltsville bee lab would be forewarned if it ever appeared in samples sent to the lab. The same holds true for other bee pests and diseases not found in the United States.

So scientists at the lab will continue to look forward to opening their mail. "Aside from the bees, you never know what you might find inside," Shimanuki says.—By **Sean Adams,** ARS.

Hachiro Shimanuki and David A. Knox are with the USDA-ARS Bee Research Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 504-8205, fax number (301) 504-8736. ◆

COP1 Protein May Spur Sprouting

When the tip of a tiny seedling pokes out of the ground into bright sunlight, profound changes take place. The hooklike tip of the little plant's skinny white "stem" slowly straightens, as if stretching to meet the sun. Miniature leaves, until now pressed together like the palms of your hands, unfold and start to grow.

These first responses to light are "some of the most dramatic redirections of development that a plant will experience during its life," says Peter H. Quail, research director at the Plant Gene Expression Center, Albany, California.

Each of the changes is the work of phytochrome—molecules that sense light. Phytochrome turns on genes that cause seeds to sprout and plants to grow.

Scientists have now discovered a protein that acts as an intermediary between phytochrome and some genes. Named COP1, the protein is likely among the first in line to receive commands from phytochrome.

"The COP1 protein probably serves as a master switch, directing genes to carry out phytochrome's commands," says Quail.

COP1 experiments may reveal new secrets about the little-known inner workings of phytochrome. And the tests may pave the way to modifying not only this protein, but also others that phytochrome influences and directs.

"We may be able to change phytochrome's instructions," notes Quail. "Perhaps tomorrow's genetic engineers could rebuild COP1 or other phytochrome-controlled proteins to create new plants that sprout, grow, and flower when people—not nature—need them to."

One possibility: futuristic plants that sprout sooner than their weed competitors. That would help crops beat weeds in the race for light, nutrients, and room to grow and would potentially reduce the need for herbicides.

JACK DYKINGA

The Plant Gene Expression Center's research director, Peter Quail, inspects mutant *Arabidopsis* plants. (K5146-16)

Quail and colleague Xing-Wang Deng, now at Yale, discovered COP1 in laboratory tests with a mustardlike plant, *Arabidopsis thaliana*. The experiments, Quail says, show that COP1 "is at the apex of a cascade of signals."

Seedlings lacking the COP1 protein offer proof of its role in their counterparts. When grown in the dark, alongside normal seeds, those missing COP1 sprouted and grew as if they were in sunlight.

Inside the normal seedlings, however, COP1 repressed precocious growth. The ordinary seedlings wouldn't open their leaves until they found daylight.

Without *Arabidopsis* to serve as a lab and greenhouse model for these and other investigations of phytochrome, says Quail, COP1 and other pieces of the phytochrome puzzle might still be missing. *Arabidopsis* plants, white-flowered and about 10 inches high when mature, grow quickly, produce an abundance of healthy seeds, and are blessed with a very small genetic makeup.

"*Arabidopsis* has about 100 times less genetic material than a wheat plant, for example," says Gerald G. Still, director of the Plant Gene Expression Center. "*Arabidopsis* has become the 'lab rat' in plant biotechnology because we can learn a lot—in short order—from it. And everything we find out from *Arabidopsis* genes could be used in genetically engineering all kinds of plants, whether they grow in a field, an orchard, or your backyard."—By **Marcia Wood**, ARS.

Peter H. Quail is at the USDA-ARS/University of California Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710. Phone (510) 559-5900, fax number (510) 559-5678. ◆

Cotton Yields Linked to Heat Tolerance

Cotton breeders have discovered a shortcut to breeding superior Pima cotton plants that could reduce the time and expense needed to develop better commercial varieties.

"During the past 40 years, plant breeders have succeeded in making Pima cotton more heat tolerant and have increased fiber yields threefold. While researchers and growers appreciated the improvement, they didn't know until recently exactly how the change came about," says John W. Radin, an Agricultural Research Service plant physiologist formerly located in the Cotton Physiology/Genetics and Host Plant Research Unit in Phoenix, Arizona.

"Our studies in growth chambers, greenhouses, and fields show that water transpiration from plant leaves has increased. Modern cotton plants give off more water when temperatures are high, as in the desert areas of Arizona and California where Pima thrives. Just as human perspiration cools the body, evaporation from leaves cools off cotton plants. Avoiding heat stress during critical growth stages is the key to high cotton yields," says Radin who conducted this research at ARS' Western Cotton Research Laboratory in Phoenix.

The results prove that cotton breeders have been unknowingly selecting for increased evaporative cooling while selecting for increased heat tolerance and yield. This finding is a big step forward: Now it may be possible to look directly at leaf temperatures and identify the genetic lines possessing heat tolerance. Crop improvement will be faster and breeders may not have to rely on repeated field-testing to confirm a new variety's heat tolerance.

"Finding a linked relationship between an agronomic trait (higher yield) with a physiological trait (increased transpiration) makes selection easier because superior plants are easily spotted in the field. Without such a link, locating plants with increased heat tolerance and higher yields requires complicated and expensive tests," says Richard G. Percy, ARS plant geneticist at the Maricopa Agricultural Center, Maricopa, Arizona, where field studies were conducted.

ARS

Pima cotton plants as seen with thermal infrared imaging during 1992 field tests in central Arizona. Colors blue through red indicate leaf temperatures ranging from 68°F to 95°F during a daytime temperature of 102°F. The blue rows represent a potentially higher yielding genotype because plants stay cooler by transpiring more water. White areas surrounding the test plot are bare soil. Recently irrigated, the plants in the test plot have cooler temperatures than those on either side. (K5156-1)

Modern varieties of Pima cotton, *Gossypium barbadense,* are better suited for desert areas than older varieties, partly because they transpire more water. Despite greater transpiration rates, modern cultivars possess a higher water use efficiency and produce more pounds of cotton per unit of water used than older, more heat-sensitive cotton varieties. This is because successive stages in the breeding program have resulted in plants with smaller leaves and greater stomatal density. Leaves from most advanced cotton lines are now about 45 percent smaller than those from a 40-year-old cultivar.

Early Pima cotton varieties yielded best when air temperatures were between 85°F and 90°F, but prevailing temperatures in the Southwest during the growing season often exceed 100°F. ARS biologist Paul J. Pinter at the U.S. Water Conservation Laboratory, Phoenix, has used modern remote sensing techniques to measure leaf temperatures. He finds that leaf temperatures of modern varieties can be as much as 10°F to 15°F cooler than the surrounding air. That's about double the temperature difference of older varieties.

This was a joint project of ARS, University of California-Los Angeles, and the Supima Association. UCLA scientists included plant physiologists Eduardo Zeiger and Zhenmin Lu.—By **Dennis Senft,** ARS.

John W. Radin is now USDA-ARS National Program Leader for plant physiology research, Rm. 336, Bldg. 005, 10300 Baltimore Ave., Beltsville, MD 20705. Phone (301) 504-6915, fax number (301) 504-5467. Richard G. Percy is at the USDA-ARS Maricopa Agricultural Center, 37860 W. Smith-Enke Rd., Maricopa, AZ 85239. Phone (602) 379-4221, fax number (602) 568-2556. Paul J. Pinter, Jr., is at the USDA-ARS U.S. Water Conservation Laboratory, 4331 E. Broadway, Phoenix, AZ 85040. Phone (602) 379-4356, fax number (602) 379-4355. ◆

Keeping Tabs on Plant Viruses and Viroids

New Tools From Genetic Engineering

The tests were positive, but the symptoms weren't.

That's when Bill Lanterman thought of asking USDA for help.

Lanterman, director of the Saanichton Plant Quarantine Station maintained by Agriculture Canada on Vancouver Island, suspected that peaches and Japanese apricots being held in the station might be infected with the devastating plum pox virus (PPV) or another unidentified virus.

Plum pox virus causes peaches, plums, apricots, and several other stone fruit trees to drop their fruit prematurely. If it stays on, the fruit is deformed and deteriorates rapidly. PPV is considered the most serious viral disease of these fruits in Europe, particularly in eastern and Mediterranean regions. And strains of the virus that attack almonds and cherries are spreading. Like most disease-causing viruses, they are carried from infected trees to healthy ones by sucking insects such as aphids.

Lanterman's suspicions were viewed with alarm, because plum pox virus doesn't occur in Canada or the United States. An accidental outbreak would wreak havoc with an industry worth a billion dollars in the United States alone.

The request for help in identification eventually came to plant pathologist Ahmed Hadidi at ARS' National Germplasm Resources Laboratory in Beltsville, Maryland. Hadidi is a world expert on plant viruses and their detection. His specialty: the detection of viral diseases of fruit trees.

He has studied viruses for 33 years and viroids for the last 18. His research has led to the discovery of both a new virus and a new viroid. Viroids are the tiniest disease-causing organism known—about 80 times smaller than the smallest virus.

Detecting Plum Pox Virus

Hadidi enlisted plant pathologist Laurene Levy to help in the search for new and better ways to test for plum pox virus in trees quarantined in the United States.

SCOTT BAUER

Always on the lookout for new viruses, plant pathologist Ramon Jordan and technician Mary Ann Guaragna collect plant samples for testing in the florist and nursery crops lab. (K5134-04)

When Levy and Hadidi tested the Canadian plants for PPV using standard detection methods like ELISA and molecular hybridization, they found that most did indeed react positively.

"In many cases, the problem with detecting PPV is that infected trees often contain very small amounts of the virus," she says, "and it's frequently below the level that current methods can detect. Added to this, the virus can be unevenly distributed throughout the tree."

To overcome these obstacles, Hadidi first analyzed published scientific findings about PPV strains. He found that this virus has unique genetic information at the end of its RNA strand, and this knowledge gave him the key to a solution: He would develop a new diagnostic test around the virus' unique genomic fingerprint.

"To make enough PPV to detect it molecularly, we used a technique developed by Hadidi to detect dapple apple viroid of pome fruit," Levy says. "It's called reverse transcription-polymerase chain reaction, or PCR."

PCR has already proven a useful tool in identifying several viruses, viroids, and mycoplasma-like organisms directly from nucleic acid extracts of infected hosts.

"The technique relies on the virus' unique end, which, like a fingerprint, distinguishes PPV strains from other plant viruses that do not affect stone fruit trees," Levy explains. "These 220 nucleotides are found next to the virus' coat protein gene.

"PCR amplifies this unique genetic end to make additional copies of the specific sequence so as to produce enough material for quick and reliable genetic fingerprinting. Amplification takes only about 3 to 5 hours," she adds.

Next, a small amount of this amplified material is spotted onto a membrane and subjected to a PPV-

specific probe—either chemiluminescent (chemical produces light to expose photographic film) or radioactive—to accurately identify it.

"What develops as dark spots on the film is PPV's fingerprint that can be compared to the standard in each test," says Levy. "The more dark spots, the more PPV."

"When we ran the new test on the Canadian plants, only a few—all coming from Asia—tested positive for PPV," Hadidi says. "The other plants were infected with a new virus that we identified and named prunus-latent potyvirus."

"It's the most accurate test to date for pinpointing PPV and for distinguishing it from other viruses," Levy says.

Besides uncovering a new virus, the test solved the mystery of the phantom virus in stone fruit that had eluded identification at the Canadian quarantine station.

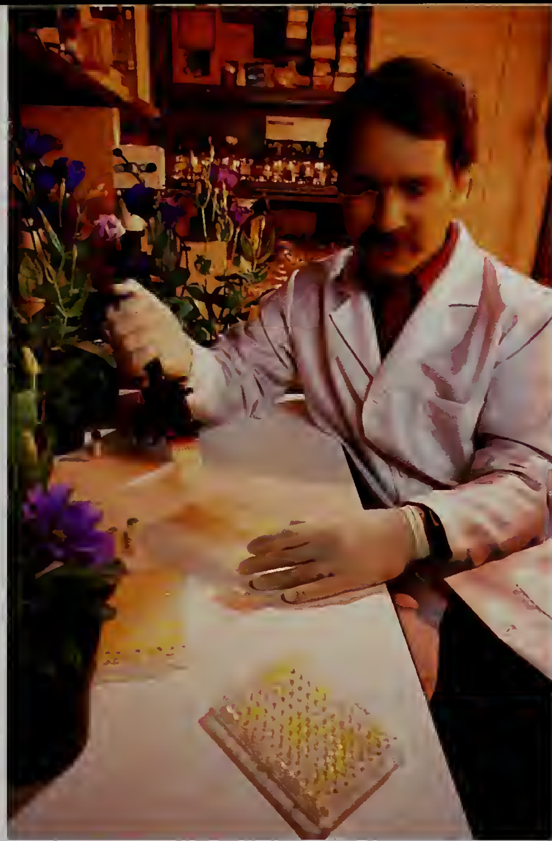
"As yet, we have no evidence that prunus-latent produces disease," says Levy, who believes the new test can be used worldwide to identify PPV in plant material.

Adds Hadidi, "This is very important, because we've always believed that PPV originated in Bulgaria. We and the Europeans may have been overlooking the virus from Asia. Now, we can no longer assume plant material from Asia is not infected with the virus."

Hadidi says the potential increase in travel and exchange of goods between the United States and Eastern Europe and the former Soviet Union heightens the risk of PPV striking American orchards.

Hadidi points out, "At present, plants must be grown for weeks or months from quarantined germplasm before seedborne diseases are revealed, whereas the new test screens plants for PPV in a day or two.

"Presently, in most countries, stone fruit seedlings and trees that react positively to PPV antiserum or molecular probes are automatically destroyed," he says. "Some plants, perhaps unnecessarily, if they are not truly infected with that virus."


SCOTT BAUER

Plant pathologist Ramon Jordan analyzes results of an ELISA test to detect potyviruses in *Lisianthus* plants. (K5133-07)

Detecting Potyviruses

PPV is just one of about nearly 180 viruses—about 36 percent of all known plant viruses—called potyviruses. The name is short for the potato virus Y group.

A polymerase chain reaction assay performed by technician Irene Danner and plant pathologist Ahmed Hadidi can detect plum pox and prunus-latent potyviruses. (K5131-18)


SCOTT BAUER

"Potyviruses make up the largest and most important group of viruses that affect U.S. crops and are responsible for estimated losses of 5 to 20 percent each year," says Roger Lawson, head of ARS' Florist and Nursery Crops Laboratory at Beltsville.

"There are no known incidents of their causing any disease in humans and animals," he adds.

These viruses are carried from plant to plant by aphids when the pests feed on leaves and stems. They also spread during harvesting and are especially devastating to the cut-flower industry.

"Unlike virus-infected food crops that lose their value but can still be used, high-value ornamental crops such as orchids can be totally lost," says Lawson. "In Florida, many commercial gladiolus crops are now almost totally infected by bean yellow mosaic virus, reducing their $13 million annual market value by as much as 15 to 20 percent."

Since 1983, plant pathologist Ramon Jordan has been working in the Florist and Nursery Crops Laboratory, looking at the structure and biochemistry of viruses to better control them. In the course of his research, he and colleague John Hammond developed monoclonal antibody-based technology for a diagnostic kit now used worldwide to detect plant-damaging potyviruses.

Says Jordan, "It will detect at least 55 distinct potyviruses that can lower the market value of vegetables and ornamentals. The kit uses the ARS-patented monoclonal antibody probe to identify the viruses that attack many plant species including beans, wheat, lettuce, and potatoes, as well as lilies, irises, and tulips."

This potyvirus kit is the first capable of detecting such a broad spectrum of plant viruses and is the first marketed under the Technology Transfer Act of 1986. That legislation

SCOTT BAUER

Using cloned probes and a specialized oven, plant pathologist Laurene Levy performs a hybridization assay to detect plum pox virus in *Prunus* germplasm. (K5131-13)

made it easier for federally funded laboratories to transfer new technologies to industry, by allowing the granting of exclusive licenses to produce or use innovative products, processes, or systems.

In 1988, Agdia, Inc., of Mishawaka, Indiana, was granted such a license to market the ARS-developed potyvirus test kit.

And Now, a New Test To Detect Viroids

Although viroids are similar to viruses in the way that they infect their host organism, they have several differences. A typical virus is made up of DNA or its chemical cousin, RNA, coiled inside a protective protein coat that helps the virus enter living cells. But viroids have no coat protein. They're just tiny bits of naked RNA.

Plant pathologist Ed Podleckis has developed a new screening test for detecting viroids that attack pome fruit trees and potatoes.

It's a faster, safer, and reliable new test that uses tissue blot. "Now, apple and pear trees coming into the United States can be tested after being grown in a greenhouse for just 2 months," Podleckis says. "Screening them for apple scar skin viroid takes just about a day or two using our new test.

"This test is 3 to 5 years quicker than the current practice of having to wait for the tree to bear fruit and looking for apple scar skin viroid symptoms like spotting and scarring of fruit to show up. And weather conditions or insect damage or other diseases could mask viroid symptoms."

Hadidi says that Podleckis' test means growers and consumers may not have to wait as long for a new fruit or vegetable variety that owes its flavor, for example, to an imported species. "We've seen prime candidates for new species, such as Chinese pears with superior flavor and market value, that spend years in quarantine."

Explains Podleckis, "A foreign species—in fact, all incoming plants—undergoes quarantine so that no viroids or other disease-causing pathogens accidently enter the country." He spent several months on developing and perfecting a new tissue blot test for detecting apple scar skin viroid.

Apple scar skin viroid infects pome fruit trees (apple, pear, and quince) in China and Japan. It's spread by grafting from cuttings. So Podleckis' apple scar skin viroid test starts with a viroid-free indicator plant to which he grafts a piece of the quarantined plant to be tested.

After the grafted plant grows for a couple of months, he takes a twig or leaf from the plant's new growth and presses it down on a wetted filter so that some of the sap sticks to it.

Podleckis describes this tissue blot test as "sort of a biochemical Dagwood sandwich made up of several layers."

The first layer is the filter and sap that are soaked in a solution containing a probe that specifically binds to any apple scar skin viroid present in the sap spot.

The probe—an RNA mirror image of apple scar skin viroid developed in the National Germplasm Resources lab by Hadidi—is labeled along its length with digoxigenin (DIG). "DIG is a steroid from a plant called *Digitalis,* a member of the foxglove family," he explains.

The next layer in the sandwich is a solution of molecules with a DIG-binding antibody at one end and the alkaline phosphatase enzyme at the other.

SCOTT BAUER

Plant pathologists Rosemarie Hammond and Ed Podleckis identify apple scar skin viroid in Virginia crabapple tissue with a tissue blot printing hybridization technique. (K5132-08)

KEITH WELLER

"Capping off the sandwich—like a layer of mustard—is a substrate solution that reacts with the enzyme to produce light in a chemiluminescent reaction. A piece of photographic film is then exposed to the membrane."

The light from the chemiluminescence exposes the film, producing a dark spot wherever apple scar skin viroid is present.

"Using tissue blots," Podleckis says, "eliminates hazardous waste now left from using organic solvents to extract nucleic acids from plant tissues. It also replaces radioactive isotopes with safer chemical probes.

"These nonradioactive probes are every bit as sensitive as the currently used radioactive ones. And they also cost less, can be reused several times, and store well frozen for up to a year."

A similar test, using a different probe, was developed by Podleckis in collaboration with Rosemarie Hammond, a plant pathologist in the Molecular Plant Pathology Laboratory in Beltsville. It is able to detect potato spindle tuber viroid that attacks potato tubers and tomato roots, stems, and leaves.

So far, Podleckis has screened over 200 apple and pear trees for apple scar skin viroid and about 100 potato plants for potato spindle tuber viroid using the new tissue blot technique. He says it has also worked, in lab experiments, for detecting other viroids in other plants.

According to Hadidi, about 25 different viroids are currently known, the first having been discovered in Beltsville in 1971 by ARS plant pathologist Theodor O. Diener. It was potato spindle tuber viroid. [See "Tracking the Elusive Viroid," *Agricultural Research,* May 1989, pp. 4-7.]—By **Hank Becker,** ARS. ◆

By modifying plants with antisense RNA, plant pathologist John Hammond has induced the virus resistance shown by the leaf on the right. (K5150-1)

Making Plants Immune to Viruses

A wide range of ornamental flowers including orchids and gladiolus—as well as some of the country's major economic crops, such as beans, peas, and forage legumes—have long needed effective protection from serious viral enemies. So plant pathologist John Hammond in the ARS Florist and Nursery Crops Laboratory at Beltsville set out to use a new genetic technology—antisense—to build in plant immunity.

"We reversed the coding sequence of a piece of genetic material taken from a plant virus to create an antisense gene," he says. "When we inserted this gene in some experimental tobacco plants, we found it 'disarmed' invading plant viruses."

Antisense technology is a form of genetic engineering in which cells are instructed to do the opposite of what one of their genes is telling them to do. Antisense is "not new, but this is the first time it's been used successfully to create virus-resistant plants," Hammond explains.

"Plants with the antisense gene will churn out antisense RNA as genetic material that binds to an invading virus' RNA. This binding apparently prevents the virus from reproducing itself in the plant. If the virus can't reproduce and spread, then it can't harm the plant."

He found that the antisense gene that was taken from bean yellow mosaic virus (BYMV) protected the engineered tobacco plants from the virus. Says Hammond, "A desert species of the tobacco plant, *Nicotiana benthamiana,* was used because it's very susceptible to potyviruses."

Hammond worked for about 2 years with plant pathologist Kathryn Kamo and plant geneticist Robert Griesbach on perfecting this technology. He believes that similar techniques should work with related viruses that infect fruit trees. And he estimates that the antisense gene might be ready in 2 to 5 years for breeders of floral and other crops to use.

Says Hammond, "BYMV-resistant plants would give growers a better chance to increase both yield and quality."

"Even more important," adds Lawson, "is the time saved. Antisense gene technology could shorten by generations the time needed to breed resistance into agronomically adapted tree crops. With traditional breeding programs, plants have to be essentially re-bred, and then even more time is needed to screen for resistance."—By **Hank Becker**, ARS.

To contact scientists mentioned in this article and sidebar, write or call Hank Becker, USDA-ARS Information Staff, 6303 Ivy Lane, Room 440, Greenbelt, MD 20770. Phone (301) 344-2769, fax number (301) 344-2311.

Will Salty Water Sabotage Almonds?

A decade of testing at Fresno, California, yields some answers.

Tomorrow's growers of crisp, flavorful almonds may find themselves in a crunch as competition for prime land and pure water heats up.

Orchardists of the future will likely have to pay more for clean water for irrigating their trees. Or, in some Western states, they may have to make do with salty, second-rate water perhaps laden with boron, selenium, and other contaminants.

To see how newly planted orchards might fare in tough times, ARS researchers and their university colleagues monitored young trees growing on clay loam soil in Central California. For the first 7 years of the 10-year test, they gave the 900 trees different amounts of salty water. Then, for the final 3 years, they gave one-third of the trees clean water, while others had to make do with the saline regimen.

Pumped from a well, the saline water carried not only sodium and calcium chloride but also a hefty load of boron, a mineral nutrient that can be extremely damaging to plants that get too much of it. The fresh water, snowmelt piped from an aqueduct, was very low in salt.

Instead of relying on flood irrigation—flooding the orchard floor several times during the spring, summer, and fall—the researchers opted for drip irrigation. With it, tubing and emitters set near trees delivered precise amounts of water daily.

CON KEYES

Plant physiologist Bob Hutmacher (right) and former ARS researcher Harry Nightingale demonstrate almond yield per tree relative to the depth of irrigation water applied during one growing season. (K5024-07)

Each year, from April through November, young trees received one of several different quantities of water. One level of irrigation, for instance, was estimated to be exactly what the trees needed to avoid water stress—the result of not getting enough water. Other levels ranged from 50 percent less than the estimated requirement, to 75 percent above it. Researchers based these calculations on an evapotranspiration coefficient, a standard measure or index of how much water an almond tree uses.

The research orchard lies about 50 miles southwest of Fresno at the University of California's West Side Field Station in the San Joaquin Valley. Orchards in the valley and elsewhere in the state produce almost all of the nation's almonds. The 1992 California almond harvest of 330,000 tons was worth about $592 million.

"We tried to approximate real-life conditions," says Robert B. Hutmacher, who is with the ARS Water Management Research Laboratory in Fresno. Former ARS researcher Harry I. Nightingale coordinated the field study, working with Hutmacher and University of California at Davis collaborators Dennis E. Rolston, James W. Biggar, Patrick H. Brown, and Douglas W. Peters.

The experiment is probably the first in the United States to scrutinize young almond trees' long-term response to salinity. Saline water in the test held more than 1,000 parts per million of dissolved salts, making it about one-thirtieth as salty as seawater.

Earlier, an ARS team at the U.S. Salinity Laboratory, Riverside, California, reported that slightly saltier water hindered growth of almond trees in greenhouses.

In the orchard study, trees reacted similarly. Though they all survived, growth rates slowed. Trees receiving all or more of their estimated water requirement as salty water, for example, put on about 20 percent less growth than trees raised elsewhere with high-quality water.

Too, nut yields were about 30 to 50 percent less than could be expected from trees of a similar age. That's why, 7 years into the experiment, the researchers chose to pipe fresh water to some of the trees. They wanted to sidestep problems—leaf damage and other injuries, for instance—that would likely accompany the buildup of salinity and boron within the trees' root zone.

"We made a choice that a lot of growers are facing right now," notes Hutmacher. "We had to decide whether to alternate good- and poor-quality water on all of the orchard during the year, or instead give some trees the good water and some other trees only the poorer. We chose the second option."

For 2 of the final 3 years of the study, nut yields were apparently influenced more by the amount of water than by its saltiness. Trees that received 100 percent or more of their water requirement produced up to twice as many nuts as those that received less.

Only in the last year of the study did trees receiving the higher levels of saline water produce fewer nuts than trees receiving matching amounts of good quality water.

In the final 2 years, leaf damage showed up on trees that had been irrigated only with saline water for nearly a decade. Leaves that were once healthy and glossy green turned yellowish, then brown. Chloride—not boron or sodium—was the likely culprit. "It built up in the root zone because there wasn't enough water to leach it away," explains Hutmacher. "If damage had continued, trees would probably have begun to lose more and more leaves. In time, they could have lost small branches, as well."

CON KEYES

More vigorous growth of almond tree on right resulted from larger applications of water even though both trees received saline water. (K5027-12)

That's what happened in an earlier study conducted by ARS and university scientists. The experiment used mature plum trees—already known to be sensitive to salty water. Irrigation water, similar in saltiness to that in the almond test, reduced plum tree growth by 40 percent after 5 years. Plum yields declined by 20 to 30 percent after 6 years.

The almond investigation suggests that even almonds—generally regarded as less sensitive to water and salt stress than plums—can't produce bountiful harvests for the long run if they're nurtured only with saline water. "You build up such toxicity levels in the soil that the yields would not be sufficient to keep you afloat economically," notes Claude J. Phene, director at the Water Management Research Laboratory.

"You can get by for the short term. But to continuously irrigate these trees with degraded water would be foolish, particularly if you're not applying enough water to move salts down beyond the root zone."

The experiment also points out that today's evapotranspiration coefficients for young almond trees underestimate their water needs. Trees that yielded the most nuts were those that received 25 to 50 percent more than the current coefficient would prescribe. Hutmacher and co-researchers hope to report more accurate coefficients in future studies.—By **Marcia Wood,** ARS.

Robert B. Hutmacher is with the USDA-ARS Water Management Research Laboratory, 2021 South Peach Avenue, Fresno, CA 93727. Phone (209) 453-3100; fax number (209) 453-3122. ◆

Cows Can Be Pretty Picky Eaters

Imagine you're at a restaurant and the waiter brings you a nice, fresh, crisp green salad. But when you look closely, you notice that there's a limp, brown, dead lettuce leaf in it. Now, wouldn't you demand a new salad?

Amazingly enough, our bovine friends are just as finicky. Range scientist David C. Ganskopp found that cattle turn up their noses at clumps of crested wheatgrass that sport as few as three dead grass stems. That wasted up to 50 percent of the available forage in an eastern Oregon study.

Plants with large amounts of this strawlike material are called wolf plants.

Ganskopp, at the ARS Range and Meadow Forage Research Unit in Burns, Oregon, says his finding means ranchers should make sure their cattle graze pastures thoroughly. Otherwise, the leftovers turn brown and unappetizing, and cattle won't go near the new, green growth surrounding them.

Grazing thoroughly doesn't mean overgrazing. "You wouldn't want to put cattle out on a pasture from April to August," says Ganskopp, "because that might entirely destroy some especially tasty species." Grazing cattle for shorter periods of time on smaller pastures—so everything gets cropped off, but no one kind of plant excessively—is a better idea.

"Or," he adds, "an alternative remedy might be to breed grasses with stems that break off easily when dry, so wolf plants wouldn't develop."

Ganskopp has uncovered another peculiar bovine eating habit: a preference for mid-sized grass clumps. When given a choice of dime- to dinner-plate-sized clumps, cattle avoided the smaller clumps and chomped mainly on those that were 3.5 to 4.5 inches in diameter, or larger.

Although it's only speculation, Ganskopp says there may be a method to the munching. Taking both tooth width and tongue length into account, he found that a 4-inch clump makes a perfect mouthful for the average Hereford that was studied.

A smaller plant would yield less grass per bite. And because of the way forage grasses grow, the biomass (green, leafy growth) yielded by larger plants in a given area is less than smaller plants provide. That's why eating mid-sized grasses is most efficient.

Ganskopp says this information should help researchers devise better management systems for cattle being fed on rangeland pastures.—By **Julie Corliss,** formerly with ARS.

David C. Ganskopp is in the USDA-ARS Range and Meadow Forage Management Research Unit, Star Rte. 1, 4.51 Hwy 205, Burns, OR 97720. Phone (503) 573-2064. ◆

Key Enzyme Linked for First Time to Corn Growth

Corn yields are reduced up to 80 percent if a key enzyme is missing, according to a U.S. Department of Agriculture scientist who says the finding is the first to link the enzyme to seed growth.

A single gene appears to control production of the enzyme, called invertase, based on preliminary studies with a mutant corn that lacks the suspected invertase-producing gene, says plant geneticist Prem S. Chourey, who is with the Agricultural Research Service in Gainesville, Florida.

Without invertase, the mutant corn's seeds are tiny, shriveled, and never fully developed.

Researchers have long known that invertase is present in corn and other plants, says Chourey. "But we didn't know exactly what role it played. We now know that it is critical for seed growth in corn, and we suspect it may play the same role in other plants."

Invertase breaks down a complex sugar, sucrose, into the simple sugars—glucose and fructose—that the seed needs to fully develop. Plant leaves produce sucrose and transport it to the seed base, the "pipeline" for carrying nutrients to the seed. But sucrose cannot pass through the base and into the seed unless it is first broken down into glucose and fructose, Chourey says.

"We aren't certain why, but we suspect that there is a structural barrier between the base and the seed that prevents sucrose from going into the seed," he says.

In studying a mutant corn, he found that it lacked invertase at the seed base. Without the enzyme to break down the sucrose, the seeds become starved and shriveled and recede from the base.

"We've shown that the seed and base communicate with each other and that invertase is the messenger between the two," Chourey says.

A better understanding of invertase, and the gene that appears to control it, may lead in the long term to genetically engineered crops with better productivity and yields.—By **Sean Adams,** ARS.

Prem S. Chourey is in the USDA-ARS Plant Stress and Protection Research Unit, University of Florida, Fifield Hall, Gainesville, FL 32611-0680. Phone (904) 392-7237, fax number (904) 392-6532. ◆

Science Update

'Charleston Hot' Ignites Pepper Passions

No more requests, please, say ARS researchers who filled more than 24,000 requests for seed samples of their new cayenne—20 times hotter than the typical cayenne—reported in the February issue of *Agricultural Research.* When Charleston Hot supplies ran out, some breeders, seed companies, farmers, and backyard pepper hobbyists had to make do with

SCOTT BAUER

Charleston Hot peppers at varying stages of maturity. (K5047-1)

Carolina Cayenne. It lacks Charleston's green-yellow-orange-red color progression, but is equally fiery. Seed for Charleston Hot will be available commercially in 1994 from South Carolina Foundation Seed, Inc., Clemson University, 1162 Cherry Rd., Clemson, SC 29631. Orders will be accepted in January. *Philip D. Dukes, U.S. Vegetable Laboratory, Charleston, South Carolina. Phone (803) 556-0840.*

Women's Energy Use Jumps During Ovulation

Women may eat more after they ovulate—to replace the extra energy they burn to maintain their basal metabolism during this part of their menstrual cycle. The dozen women volunteers in an ARS study burned 5 to 7 percent more calories in the interval between ovulation and menstruation than they did during or after menstruation. That means researchers would more accurately measure women's energy expenditure by measuring them at the same points in the cycle or by accounting for cyclic differences in metabolism. *Juliette Howe, Energy and Protein Nutrition Laboratory, Beltsville, Maryland. Phone (301) 504-8181.*

New Grass for Cattle Means More Seed for Growers

Bison, a new buffalograss for grazing cattle, has a bonus along with the same growth and grazing benefits of the popular Texoka variety. Bison yielded 24 percent more live seed in a 2-year Oklahoma test—a plus for making additional plantings. Bred for the southern half of the Great Plains, Bison was developed by ARS and Oklahoma State University. *Timothy L. Springer, South Central Family Farms Research Center, Booneville, Arkansas. Phone (501) 675-3834.*

Bacteria To Make Egg-Stopping Hormone in Mosquitoes?

ARS and University of Florida scientists are pursuing a new, high-tech way of making mosquitoes buzz off. They found that a synthetic version of the female mosquito's oostatic hormone blocks an enzyme she needs to produce viable eggs. The scientists will try to insert the hormone-making gene into bacteria that infect only mosquitoes. *David A. Carlson, Medical and Veterinary Entomology Research Laboratory, Gainesville, Florida. Phone (904) 374-5929.*

Fungi Among the Whiteflies

Natural outbreaks of a fuzzy fungus are smothering sweetpotato whiteflies in some Texas and California vegetable and cotton fields. ARS scientists are working with a Montana company, Mycotech, to see if the fungus, a *Paecilomyces* species, can be mass-produced as a whitefly control. Fungal spores kill whiteflies in about 5 days. *Raymond I. Carruthers, Biological Pest Control Research, Weslaco, Texas. Phone (210) 969-4852.*

ARS Finishes New Erosion Yardstick

This fall, USDA's Soil Conservation Service will begin using ARS' computerized Revised Universal Soil Loss Equation. The equation will supply farmers with more accurate information on how to reduce erosion by water. The original USLE, developed in 1958, has long been a primary tool in conservation planning worldwide. *Kenneth G. Renard, Southwest Watershed Research, Tucson, Arizona. Phone (602) 670-6481.*

ARS, NIH Join To Gather Data on Carotenoids

Anticancer value of beta carotene and other carotenoids will be easier to assess with a new database developed by ARS and the National Cancer Institute. Available from NCI, the database gives levels of the five most common carotenoids in 150 fruits and vegetables and more than 2,000 mixed foods containing fruits or vegetables. Carotenoids, a group of nearly 600 pigments, give many foods their natural yellow, orange, and red colors. Green leafy vegetables, too, have carotenoids aplenty, but they're masked by chlorophyll. *Gary R. Beecher and Joanne M. Holden, Nutrient Composition Laboratory, Beltsville, Maryland. Phone (301) 504-8356/8186.*

U.S. Department of Agriculture
Agricultural Research Service
Room 408
6303 Ivy Lane
Greenbelt, MD 20770

Official Business
Penalty for Private Use $300

To stop mailing ______________ ☐
To change your address ________ ☐
Please return the mailing label from this magazine.

Bulk Rate
Postage and Fees Paid
U.S. Department of Agriculture
Permit No. G-95

Superintendent of Documents Subscription Order Form

Order Processing Code:
6130

Charge your order. It's Easy! MasterCard VISA

To fax your orders (202) 512 2233

☐ **YES,** enter my subscription as follows:

___subscriptions to **Agricultural Research** for $28.00 per year ($35.00 foreign).

The total cost of my order is $__________. Price includes regular domestic postage and handling and is subject to change.

Call Order and Information Desk at (202) 783-3238 after 8/31/93 to verify prices.

(Company or personal name) (Please type or print)

(Additional address/attention line)

(Street address)

(City, state, ZIP Code)

(Daytime phone including area code)

(Purchase order No.)

May we make your name/address available to other mailers? YES ☐ NO ☐

Please choose method of payment:

☐ Check payable to the Superintendent of Documents
☐ GPO Deposit Account ☐☐☐☐☐☐☐-☐
☐ VISA or MasterCard Account

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐☐☐☐
Exp. date

(Authorizing Signature)

Mail To: New Orders, Superintendent of Documents
P.O. Box 371954, Pittsburgh, PA 15250-7954

Upcoming in the August Issue

☛ ARS hydrologist Doug Boyer turns to spelunking to get an underground view of agricultural pollution. "Caves are Nature's drainage pipes," he says.

☛ A natural yeast mixed with the wax that normally seals moisture in fruit may be a great way to prevent gray mold from developing. ARS scientists at the Subtropical Horticulture Research Laboratory in Miami are testing it on grapefruit.

☛ An ARS computer program points out the life-saving benefits of using insect repellants and insecticides by U.S. troops in Somalia.